

Applicant(s): David A. Sanders et al.

Serial No. 09/762,224

Int'l Filing Date: 4 August 1999

For: PSEUDOTYPED RETROVIRUSES AND STABLE CELL LINES FOR THEIR PRODUCTION

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**Remarks**

The specification, as filed, incorrectly designated the nucleotide sequence and the amino acid sequence encoded by this nucleotide sequence with a single SEQ ID No.:. The present preliminary amendment corrects this misnumbering. The Preliminary Amendment also corrects a typographical error in a citation at page 10, line 30 of the specification.

**Sequence Listing**

In response to the Notification of Defective Response mailed 8 January 2003, enclosed is a substitute sequence listing in written and computer readable forms. The substitute sequence listing reflects a change in file reference number at field <130> and a correction in the spelling of inventor Fischbach's name in field <110>.

In accordance with Rule §1.821(e), the information recorded in computer readable form is identical to the written substitute sequence listing. Furthermore it is submitted that the sequence listing includes no new matter.

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**Conclusion**

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

**CERTIFICATE UNDER 37 C.F.R. 1.10:**

The undersigned hereby certifies that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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Date of Deposit: 10 February 2003

Respectfully submitted for  
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**APPENDIX A - SPECIFICATION AMENDMENTS  
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

**Serial No.: 09/762,224**

**Docket No.: 290.00490101**

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Amendments to the following are indicated by underlining what has been added and shading what has been deleted.

**In the Specification**

The paragraph beginning at page 10, line 24, has been amended as follows:

The retroviral *gag*, *pro* and *pol* nucleotide sequences, and other retroviral nucleotide sequences for forming the specified pseudotyped retroviruses, may be obtained from a wide variety of genera in the family Retroviridae, including, for example, Oncoviruses, including Oncovirus A, B, C and D, lentiviruses and spumavirus F. Such sequences are preferably obtained from the Moloney murine leukemia virus (MMLV; in the genus Oncovirus C). Such sequences are well known in the art. For example, nucleotide sequences encoding MMLV *gag*, *pro* and *pol* may be found in Van Bereven et al. *Cell* (1981)27:97-108. Most preferably, such sequences are obtained from lentiviruses. Unlike most retroviruses, lentiviruses have the capacity to integrate the genetic material they carry into the chromosomes of non-dividing cells as well as dividing cells. Therefore, lentiviral nucleotide sequences encoding proteins that allow for chromosomal integration of virally transported nucleic acid in non-dividing cells are advantageously employed, as the host range of the pseudotyped retroviruses will be broadened.

The paragraph beginning at page 13, line 11, has been amended as follows:

In one form of the present invention, the cells include nucleotide sequences encoding glycoproteins from an alphavirus. In a most preferred embodiment, the cells include nucleotide sequences encoding glycoproteins from the viral species Ross River (depicted in SEQ ID NO:1 and SEQ ID NO:2 ~~SEQ ID 1~~). The viral transmembrane glycoprotein complex that is responsible for the binding of the alphavirus to the surface of a susceptible cell and for the fusion of the viral and cellular membranes that occurs during the process of viral entry includes a trimer of a heterodimer of two transmembrane proteins, which are denoted E<sub>1</sub> and E<sub>2</sub> and which are

encoded by an E<sub>3</sub>-E<sub>2</sub>-6K-E<sub>1</sub> glycoprotein coding region (E<sub>3</sub> and 6K refer to viral proteins involved in maturation of E<sub>1</sub> and E<sub>2</sub> as known in the art) on the alphaviral genome. The E<sub>2</sub>-E<sub>1</sub> coding region includes an E<sub>3</sub> glycoprotein coding region as well as the 6K protein coding region. Such nucleotide sequences may be obtained by methods known to the skilled artisan as discussed for the *gag*, *pro* and *pol* nucleotide sequences above. For example, the E<sub>2</sub>-E<sub>1</sub> coding region may be obtained as discussed in Kuhn et al. (1991) *Virology* 182:430-441. The E<sub>2</sub>-E<sub>1</sub> glycoprotein coding region is also operably linked to a promoter sequences, such as described above, at its 5' end.

The paragraph beginning at page 14, line 9, has been amended as follows:

In another embodiment, the cells include nucleotide sequences encoding glycoproteins from a filovirus. Such filoviruses also exhibit a broad host range. A wide variety of nucleotide sequences that encode filoviral glycoproteins may be used to produce the inventive cells of the present invention. For example, nucleotide sequences encoding glycoproteins from the Marburg and Ebola virus (in the family Filoviridae and, including, for example, Ebola-Zaire and Ebola-Reston) may be introduced into the cells described above to produce a pseudotyped retrovirus. SEQ ID NO:3 **SEQ ID 2** shows the Ebola Zaire glycoprotein-encoding sequence and SEQ ID NO:5 **SEQ ID 3** shows the Marburg virus glycoprotein-encoding sequence. The nucleotide sequences encoding the filoviral glycoproteins may be obtained as described in Sanchez et al. (1993) *Virus Res.* 29(3):215-240 and Will et al., (1993) *J. Virol.* 67:1203-1210. Moreover, such sequences may be obtained by other methods known to those skilled in the art, as described above for the togaviruses.

The paragraph beginning at page 37, line 17, has been amended as follows:

pEZGP1 was produced by cloning into the polylinker of plasmid pcDNA3 nucleotide sequences corresponding to nucleotides 6029-8253 [sequences 6029-8253, corresponding to nucleotides 132-2354 described in Genbank as Accession Number U23187, are shown in SEQ ID NO:3 **SEQ ID 2** from the Ebola Zaire virus genome, with the exception that an

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additional "a" has been inserted between nucleotides 1027 and 1028 in SEQ ID NO:3 **SEQ ID 2** compared to the Genbank sequence] from the complete Ebola Zaire genome [described in Sanchez et al. (1993) *Virus Res.* 29(3):215-240] obtained by digestion of the MP1153 plasmid provided by Dr. Anthony Sanchez with Eco RI and HindIII. SEQ ID NO:4 **SEQ ID 2 also** shows the amino acid sequence of the Ebola Zaire glycoprotein.